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Washington, DC

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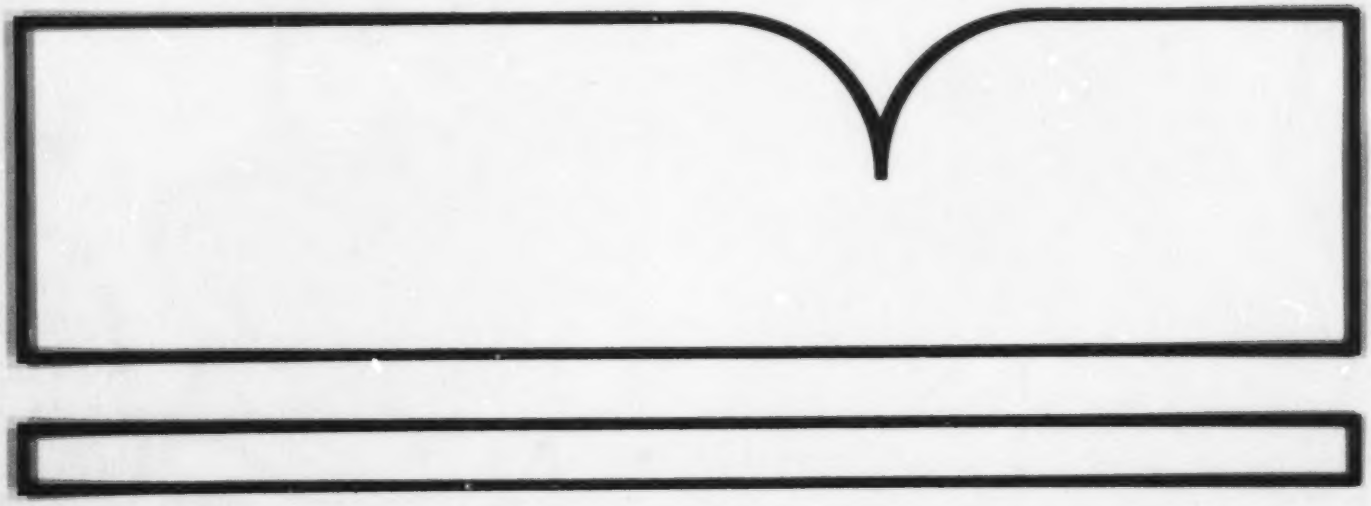

U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-248176

PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION E
Addendum 1
HAZARD EVALUATION:

WILDLIFE AND AQUATIC ORGANISMS

Series 71-2

Acute Dietary LC_{50} Test for Waterfowl and Upland Game Birds,

Series 71-1

Acute Oral LD_{50} Test for Waterfowl or Upland Game Birds,

AND

Series 71-4

Avian Reproduction Test for Waterfowl and Upland Game Birds

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Office of Pesticide Programs
U.S. Environmental Protection Agency

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Addendum 1 to Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms
(EPA-540/9-82-024 and NTIS Order Number PB83-153908) October 1982

16. Abstract (Limit: 200 words)
The Data Reporting Guideline (DRG) for Acute Dietary LC50 and Acute Oral LD50 Tests for Waterfowl and Upland Game Birds and Avian Reproduction Tests gives guidance to the pesticide registrant on the format of the study report so that the Agency can review it efficiently. This guidance clarifies sections in the existing Pesticide Assessment Guidelines. Subdivision E, Series 71-1, 71-2, and 71-4 on data reporting provides an outline for the study report and formats for data tables which data submitters can use in preparing their reports to be submitted to EPA to meet 40 CFR 158 data requirements for the registration of pesticides.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement: Unclassified and freely available	19. Security Class (This Report): Unclassified	21. No. of Pages: 46
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(See ANSI-Z39.18) See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce

Subdivision E - Acute Dietary LC_{50} Test for Waterfowl and Upland Game Birds

Table of Contents of Addendum

Discussion	Page
Introduction	i
Response to Public Comments	i
Guideline	
Title/Cover Page	1
Table of Contents	1
I. Summary/Introduction	1
II. Materials/Methods	2
III. Statistical Analysis	5
IV. Results/Discussion	5
V. Conclusion	5
VI. Tables/Figures	5
VII. Appendix(es)	5

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Acute Dietary LC_{50} Test for Waterfowl and Upland Game Birds

Subdivision E, Series 71-2

DATA REPORTING

INTRODUCTION

A. Purpose

To provide a data reporting format for a study of an acute dietary LC_{50} for waterfowl or upland game birds.

B. Objective

This Data Reporting Guideline is designed to aid the petitioner/registrant in generating reports which are compatible with the Agency's review process. While following this Guideline is not mandatory, data submitters are encouraged to submit complete reports which can be efficiently reviewed by the Agency.

RESPONSE TO PUBLIC COMMENTS

The purpose of this section is to acknowledge and address the concerns expressed in the letters of comment received by the Agency in reponse to the public notice in Federal Register Volume 50, No. 147, p. 31010, July 31, 1985.

This addendum to the Pesticide Assessment Guidelines [Subdivision E] is to be considered an all-encompassing document. The Ecological Effects Branch (EEB) has reviewed the 22 comments submitted by the registrants and/or committees in regard to the Data Reporting Guideline (DRG). This Guideline is not intended to create new data requirements, but to provide for consistent reporting of the necessary avian data required to perform a scientifically sound hazard assessment. This approach should eliminate most, if not all, of the recycling of submissions between EEB scientists and the registrant.

The submitted comments pertain to either items common to all studies or a study specific item. Hence, this discussion has been arranged to consider the general comments first, followed by the specific comments.

General Comments

1. Comments on the location of a specific reporting item -

Considering the need to maintain a consistent format, revisions were made to the DRG in response to these comments wherever possible.

2. Report of the impurities of the test substance -

   Under the present Guideline, these data are required under 158.110 and are reported to the organization reviewing product chemistry submissions. Hence, these data are not reported directly to reviewers of ecological effects studies. Therefore, EEB included item II(A)1.f in this DRG as an option for the registrant. By doing this, a company may avoid any delays which may inadvertently occur in our efforts to obtain the data. To further assure that this item will not be mistaken as a requirement, an explanation has been added to the Guideline.

3. Ingredient information from testing laboratories -

   It appears some sponsors do not make this information available to testing laboratories. In such a situation, the sponsor bears the responsibility since he is submitting the data for registration.

4. Standard evaluation procedures (SEPs) -

   SEPs are guidance documents which explain the procedures used to evaluate environmental effects data submitted to the Office of Pesticide Programs. They are also available from the National Technical Information Service. This DRG is compatible with the Agency's review procedures.

5. Control data -

   The DRG has provided space for reporting results for both control and solvent control results. Both types of controls are commonly reported to the EPA and are also incorporated into the SEPs.

6. Due to the comments, the following items have been deleted from the DRG:

   A. Chemical abstract number or code;
   B. EPA registration number;
   C. Age of material;
   D. Blood testing for a particular test hatch;
   E. Histopathology;
   F. Arrangement of cages in relation to the treatment levels.

7. Environmental conditions -

   Each study reporting item was revised, following the Guideline and SEPs.

8. Pretest diet -

   This is required under §70-4(c)(D) of the Guideline.

Study Specific Comments

1. The following were deleted as a result of comments:

   A. Sex: Sex ratio;
   B. Fasting period;
   C. Method of identifying each bird; and
   D. Regurgitation as a specific symptom.

2. Health of birds -

   This item was retained since it was mentioned in the Guideline §71-2(d)(i) under "Health of Birds." Also, it is included in the SEPs.

3. Food consumption and body weight -

   One comment was made concerning the crowding of all the data onto one table. The proposed table is a minimum of information expected. The data may be expanded to two tables if necessary.

4. Cumulative mortalities of controls (Table 2) -

   A control for each dosage level is frequently received. Hence, the table was made to accommodate these studies as well as those with only one control level (see new footnote under Table 2).

5. Graphs, printouts, and other calculations -

   These items are not required by the Guideline or the SEPs. Hence, the statement was reworded to emphasize that such items are optional.

GUIDELINE

The following describes the order and format for a study report.

TITLE/COVER PAGE

Title/cover page and other documents preceeding the study report are discussed in FR 49, 37930.

TABLE OF CONTENTS

I. SUMMARY/INTRODUCTION

A. Sponsor (name of study owner);

B. Name(s) of principal investigator(s);

C. Location of study;

D. Location of raw data and final report;

E. Material (common/trade name);

F. Subject (final or draft report, acute dietary LC_{50} - species tested);

G. Test doses (control, concurrent vehicle control,#,#,#,#,# ppm);

H. Test dates (initiation - date started on diet, termination - date of last day of observation);

I. Length of study (days);

J. Results (LC_{50} value, 95% confidence limits and no-observed-effect level);

K. Tested material (chemical name, formulation, percent active ingredient);

L. Test species/strain (both common and scientific name);

M. Source of birds (company, address);

N. Age of birds at initiation of study (days).

II. MATERIALS/METHODS

This section is a narrative which should include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or the sponsor).

   a. Chemical name;

   b. Composition (qualitative and quantitative description);

   c. Molecular structure;

   d. Percent active ingredient; and

   e. Source, lot number or code.

2. Preparation of test diets (control/treatment).

   a. Any vehicle used to dissolve or dilute the test substance with the feed;

   b. Percent vehicle used by weight relative to the feed weight;

   c. Amount of vehicle added to control diet;

   d. Description of test diet and any other ingredients such as antibiotics, vitamins, or food additives which may have been in the feed preceding or during testing;

   e. Water and feed source;

   f. Type of mixer and method of mixing in the test material and vehicle;

   g. Report data if food was analyzed for the pesticide concentration;

   h. Total volume of material administered (test substance plus carrier) in feed; and

   i. Method used to determine concentration levels.

B. Test Birds

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E.

2. Breeding History.

   a. State if test birds:

      (1) Phenotypically similar to wild counterpart;

      (2) All from the same hatch; and

      (3) Pen-reared.

   b. History of colony:

      (1) Observed diseases; and

      (2) Disease treatments.

3. Description of pretest conditions.

   a. Health:

      (1) Sickness;

      (2) Injuries;

      (3) Abnormalities; and

      (4) Mortality.

   b. Describe pretest diet (identification and composition including any antibiotics, vitamins, or food additives);

   c. Housing conditions:

      (1) Number of animals per cage;

      (2) Ambient temperature and humidity;

      (3) Photoperiod and lighting; and

      (4) Source of feed and water.

C. Methods

1. Pen Facilities:

   a. Dimensions of test pen(s) (cm);

   b. Temperature (C°) and relative humidity;

   c. Photoperiod and lighting;

   d. Pen locations (indoor or outdoor);

   e. Pen construction materials;

f. Availability of water throughout the test; and

g. Provisions for ventilation.

2. Concentration and dosage mortality/effects data.

a. Method of identifying treatment levels;

b. Randomization plan for treatments, if performed;

c. Rationale for selection of method, route, or frequency, if different from that recommended in Subdivision E;

d. A description of the steps taken to maintain the concentration(s), if the test substance is administered in the feed. Data from subdivisions other than E, such as Subdivision O or N, suggest that the actual concentrations over the length of the test may decrease by 30 percent or more.

e. Number of concentrations;

f. Number of days on test material;

g. Number of days on food free of test material;

h. Number of birds per cage;

i. Number of birds per concentration and control group(s); and

j. Method for assigning test organisms to test and control groups.

3. Bird body weights or other physiological measurements (describe methods, see also Tables 3 and 4 for reporting results).

4. Food consumption - collection methods (see Tables 3 and 4 for reporting results):

a. Schedule; and

b. Provisions for minimizing food spillage and preventing air contamination by volatile chemicals.

5. Observations:

a. Frequency, duration, and method of observations;

b. Signs of intoxication and abnormal behavior;

c. Palatability or repellency;

d. List tissue necropsies, if performed;

e. Criteria for determining effects; and

f. Define technical terminology used to describe signs of intoxication.

III. STATISTICAL ANALYSIS

A. Cite references (author, title, journal, number, page, etc.); and

B. Rationale for deviations from the cited method.

IV. RESULTS/DISCUSSION

A. LC_{50} in mg/kg with 95% confidence limits;
Slope of the dose response line, if the method provides the slope;
No-observed-effect level;

B. Any gross pathological changes as noted by gross necropsy examination, including characterization of gross lesions;

C. Detailed description of the nature, incidence, time of occurrence, severity, and duration of all observed toxic effects, including death and any other abnormal or unusual signs and symptoms. For example, report any specific signs of intoxication associated with death and the time of onset and remission of toxic signs. Also report marked observations of fecal material and urine.

D. Report the results of residue analysis of the test diets, if performed; and

E. Report any observations indicating repellency or palatability problems.

V. CONCLUSION

This is a summation of the above Results/Discussion section.

VI. TABLES/FIGURES

VII. APPENDIX(ES)

This is optional. You may attach graphs, raw data, printouts, calculations, etc., which may further support the study.

TABLE 1

CUMULATIVE MORTALITIES OF (Test species name)

EXPOSED TO (Chemical) FOR FIVE DAYS

Concentration	Number Dead/Number Exposed Day of Death								
ppm	0	1	2	3	4	5	6	7	8
xx	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x
xx	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x
xx	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x
xx	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x
xx	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x

The LC_{50} value was determined to be _____ ppm.

_____

TABLE 2

CUMULATIVE MORTALITIES OF (Test species name)

Concentration	Number Dead/Number Exposed Day of Death								
ppm	0	1	2	3	4	5	6	7	8
0	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x
0*	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x
0*	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x
0*	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x
0*	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x	x/x

_____

* Studies are not required to provide a control to match each dosage level. However, this table has allowed space for studies with this type of study design.

TABLE 3

BODY WEIGHT AND ESTIMATED FEED CONSUMPTION OF (Test species name)

EXPOSED TO (Chemical) FOR FIVE DAYS

Material	Conc. ppm	Average Body Weight (g) Day 0	Day 5	Day 8	Feed Consumption* Days 0-5	Days 6-8	F.C./Bird/Day Days 0-5	Days 6-8
(Chemical's common name)	x	xx	xx	xx	xxx	xxx	x	x
	x	xx	xx	xx	xxx	xxx	x	x
	x	xx	xx	xx	xxx	xxx	x	x
	x	xx	xx	xx	xxx	xxx	x	x
	x	xx	xx	xx	xxx	xxx	x	x

* Total estimated feed consumption (g).

TABLE 4

BODY WEIGHT AND ESTIMATED FEED CONSUMPTION OF (Test species name)

Material	Conc. ppm	Average Body Weight (g) Day 0	Day 5	Day 8	Feed Consumption* Days 0-5	Days 6-8	F.C./Bird/Day Days 0-5	Days 6-8
CONTROL	0	xx	xx	xx	xxx	xxx	x	x
	0	xx	xx	xx	xxx	xxx	x	x
	0	xx	xx	xx	xxx	xxx	x	x
	0	xx	xx	xx	xxx	xxx	x	x
	0	xx	xx	xx	xxx	xxx	x	x

* Total estimated feed consumption (g).

Subdivision E - Acute Oral LD_{50} Test for Waterfowl or Upland Game Birds

Table of Contents of Addendum

Discussion	Page
Introduction	i
Response to Public Comments	i
Guideline	
Title/Cover Page	1
Table of Contents	1
I. Summary/Introduction	1
II. Materials/Methods	2
III. Results/Discussion	5
IV. Conclusion	5
V. Tables/Figures	5
VI. Appendix(es)	5

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Acute Oral LD_{50} Test for Waterfowl or Upland Game Birds

Subdivision E, Series 71-1

DATA REPORTING

INTRODUCTION

A. Purpose

To provide a data reporting format for a study of an acute oral LD_{50} for waterfowl or upland game birds.

B. Objective

This Data Reporting Guideline is designed to aid the petitioner/registrant in generating reports which are compatible with the Agency's review process. While following this Guideline is not mandatory, data submitters are encouraged to submit complete reports which can be efficiently reviewed by the Agency.

RESPONSE TO PUBLIC COMMENTS

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General Comments

1. Comments on the location of a specific reporting item -

Considering the need to maintain a consistent format, revisions were made to the DRG in response to these comments wherever possible.

2. Report of the impurities of the test substance -

   Under the present Guideline, these data are required under 158.110 and are reported to the organization reviewing product chemistry submissions. Hence, these data are not reported directly to reviewers of ecological effects studies. Therefore, EEB included item II(A)1.f in this DRG as an option for the registrant. By doing this, a company may avoid any delays which may inadvertently occur in our efforts to obtain the data. To further assure that this item will not be mistaken as a requirement, an explanation has been added to the Guideline.

3. Ingredient information from testing laboratories -

   It appears some sponsors do not make this information available to testing laboratories. In such a situation, the sponsor bears the responsibility since he is submitting the data for registration.

4. Standard evaluation procedures (SEPs) -

   SEPs are guidance documents which explain the procedures used to evaluate environmental effects data submitted to the Office of Pesticide Programs. They are also available from the National Technical Information Service. This DRG is compatible with the Agency's review procedures.

5. Control data -

   The DRG has provided space for reporting results for both control and solvent control results. Both types of controls are commonly reported to the EPA and are also incorporated into the SEPs.

6. Due to the comments, the following items have been deleted from the DRG:

   A. Chemical abstract number or code;
   B. EPA registration number;
   C. Age of material;
   D Blood testing for a particular test batch;
   E. Histopathology;
   F. Arrangement of cages in relation to the treatment levels.

7. Environmental conditions -

   Each study reporting item was revised, following the Guideline and SEPs.

8. Pretest diet -

   This is required under §70-4(c)(D) of the Guideline.

Study Specific Comments

1. Necropsy and pathology -

   These items are required under 71-1(c)(19) of the Guideline.

2. These items are provided in anticipation of the new Guideline:

   Bird weighings on Day 1 and 14.

3. Table 1 - Dose Calculations -

   This table was simplified by deleting the percent of vehicle/bird. The remaining items are required under the SEP on page 4.

4. Graphs, printouts, and other calculations -

   These items are not required by the Guideline or the SEPs. Hence, the statement was reworded to emphasize that such items are optional.

GUIDELINE

The following describes the order and format for a study report.

TITLE/COVER PAGE

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A. Sponsor (name of study owner);

B. Name(s) of principal investigator(s);

C. Location of study;

D. Location of raw data and final report;

E. Material (common/trade name);

F. Subject (final or draft report, acute oral LD_{50} - species tested);

G. Test doses (control, concurrent vehicle control,#,#,#,#,# mg/kg);

H. Test dates (initiation - date dosed, termination - date of last day of observation);

I. Length of study (days);

J. Results (LD_{50} value, 95% confidence limits and no-observed-effect level);

K. Tested material (chemical name, formulation, percent active ingredient);

L. Test species/strain (both common and scientific name);

M. Source of birds (company, address);

N. Age at initiation of study (days).

II. MATERIALS/METHODS

This section is a narrative which should include the following items.

A. Test Substance

1. Identification (these items may be submitted by the laboratory or the sponsor).

   a. Chemical name;

   b. Composition (qualitative and quantitative description);

   c. Molecule structure;

   d. Percent active ingredient; and

   e. Source, lot number or code.

2. Preparation of test diets (control/treatment).

   a. Report vehicle used to dissolve or dilute the test substance or used to mix the test substance with the feed;

   b. Describe the preparation methods.

B. Test Birds

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E.

2. Sex (describe method of selection and give sex ratio).

3. Breeding history.

   a. History of colony:

      (1) Observed diseases or other medical problems; and

      (2) Disease treatments.

   b. State if test birds:

      (1) All from the same hatch;

      (2) Pen-reared;

      (3) Previously mated or unmated;

      (4) Phenotypically similar to wild counterpart; and

(5) In a reproductive state brought on artificially, identify the individuals.

4. Pretest period.

a. Acclimation period (days);

b. Describe pretest diet (identification and composition including any antibiotics, vitamins, or food additives);

c. Housing conditions:

(1) Number of animals per cage;

(2) Ambient temperature and humidity;

(3) Photoperiod and lighting; and

(4) Source of feed and water.

d. Health:

(1) Mortality;

(2) Sickness; and

(3) Weight loss.

e. Fasting period (hours).

C. Dosage

1. Amount of test material dosed per bird (see Table 1).

2. Method of administration (vehicle control/treatment).

a. Oral intubation; and

b. Capsule (describe size and type, if used).

3. Rationale for selection of method, route, or frequency, if different from that recommended in Subdivision E.

D. Methods

1. Number of treatment levels;

2. Number of birds per treatment levels;

3. Method of assigning birds to treatment levels and control groups;

4. Method used to determine the treatment levels;

5. Size of cages (height, width, length-cm);

6. Number of birds per cage;

7. Number of controls;

8. Type of controls (e.g., solvent, carrier, negative or positive);

9. Pen location (indoor or outdoor); and

10. Environmental conditions:

    a. Pens indoor- ambient temperature, humidity, photoperiod and lighting; and

    b. Pens outdoor- weather conditions.

11. Source and availability of feed and water;

12. Diet description and any antibiotics, vitamins, or food additives added to the feed;

13. Schedule and methods for gross necropsy examinations, if performed;

14. List tissue necropsies, if performed;

15. Length of the total observation period (number of days);

16. Frequency and duration of each observation;

17. Bird weights (report when and how determined, see Table 2 for reporting results);

18. Food consumption data for treated and control birds (see Table 2 for reporting results);

19. Define technical terminology used to describe signs of intoxication; and

20. Define criteria for determining effects.

E. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.); and

2. Rationale for deviations from the cited method.

III. RESULTS/DISCUSSION

A. LD_{50} in mg/kg with 95% confidence limits;
No-observed-effect level;
Slope of the dose response line, if the method provides a slope.

B. Detailed description of the nature, incidence, time of occurrence, severity, and duration of all observed toxic effects, including death (see Table 3) and any other abnormal or unusual signs and symptoms. For example, report any specific signs of intoxication associated with death and the time of onset and remission of toxic signs. Also report marked observations of fecal material and urine.

C. Gross pathological changes as noted by gross necropsy examination;

D. Describe control bird parameters, behavior, and general health which appear atypical;

E. Indicate the test procedures which deviate from the EPA Guideline and provide a rationale for the change; and

F. Report any other items related to the study which may be of value in hazard assessment or evaluation of the study, e.g. regurgitaton.

IV. CONCLUSION

This is a summation of the above Results/Discussion section.

V. TABLES/FIGURES

VI. APPENDIX(ES)

This is optional. You may attach graphs, raw data, printouts, calculations, etc., which may further support the study.

TABLE 1
DOSE CALCULATIONS
(Common Chemical Name)

The following Stock Solutions were prepared:

Stock Solution #2 # grams (test material)/Q.S. # mls. (vehicle)
Stock Solution #3 # grams (test material)/Q.S. # mls. (vehicle)
Stock Solution #4 # grams (test material)/Q.S. # mls. (vehicle)

The birds were individually weighed and dosed as follows:

Dosage mg/kg		1	2	3	4	5	6	7	8	9	10
Vehicle Control	Wt. of Bird (g)	x	x	x	x	x	x	x	x	x	x
	Amount of Vehicle(g)	x	x	x	x	x	x	x	x	x	x
	% of Vehicle/Bird(g)	x	x	x	x	x	x	x	x	x	x
xxxx	Wt. of Bird (g)	x	x	x	x	x	x	x	x	x	x
	Total Volume Of St. Sol. #4 (ml)	x	x	x	x	x	x	x	x	x	x
	Amount of Exp. Material (g)	x	x	x	x	x	x	x	x	x	x
xxxx	Wt. of Bird (g)	x	x	x	x	x	x	x	x	x	x
	Total Volume of St. Sol. #4 ml)	x	x	x	x	x	x	x	x	x	x
	Amount of Exp. Material (g)	x	x	x	x	x	x	x	x	x	x

(continued)

TABLE 1

DOSE CALCULATIONS (CONTINUED)

Dosage mg/kg		1	2	3	4	5	6	7	8	9	10
xxxx	Wt. of Bird (g)	x	x	x	x	x	x	x	x	x	x
	Total Volume Of St. Sol. #3 (ml)	x	x	x	x	x	x	x	x	x	x
	Amount of Exp. Material (g)	x	x	x	x	x	x	x	x	x	x
xxxx	Wt. of Bird (g)	x	x	x	x	x	x	x	x	x	x
	Total Volume Of St. Sol. #3 (ml)	x	x	x	x	x	x	x	x	x	x
	Amount of Exp. Material (g)	x	x	x	x	x	x	x	x	x	x
xxxx	Wt. Of Bird (g)	x	x	x	x	x	x	x	x	x	x
	Total Volume of St. Sol. #2 (ml)	x	x	x	x	x	x	x	x	x	x
	Amount of Exp. Material (g)	x	x	x	x	x	x	x	x	x	x

Table 2

Food Consumption and
Average Body Weight (g)

(Chemical Common Name)

Material	Dosage mg/kg	Average Body Weight (g)**		Estimated Food Consumption Per Bird Per Day**	
		Day 1	Day 14	1-7 (g)	8-14 (g)
(chemical's common name)	x	x	x	x	x
	x	x	x	x	x
	x	x	x	x	x
	x	x	x	x	x
	x	x	x	x	x
Controls	x	x	x	x	x
	x	x	x	x	x
	x	x	x	x	x
	x	x	x	x	x
	x	x	x	x	x

* Data not available due to total mortality.
** Additional data may be inserted if the study is extended.

TABLE 3

DOSE MORTALITY
(Common Chemical Name)

Chemical Dosage mg/kg	Time of Death, Day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	x	x	x	x	x	x	x	x	x	x	x	x	x	x

LD_{50} is___mg/kg, confidence limits (95%)____to____mg/kg.

Control/ Vehicle * Control Dosage mg/kg	Time of Death, Day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	x**	x	x	x	x	x	x	x	x	x	x	x	x	x
	x**	x	x	x	x	x	x	x	x	x	x	x	x	x
	x**	x	x	x	x	x	x	x	x	x	x	x	x	x
	x**	x	x	x	x	x	x	x	x	x	x	x	x	x

*If both control and vehicle control are performed provide data in this format for both.
**Location of additional control/vehicle control levels, if performed.

Subdivision E - Avian Reproduction Test for Waterfowl and Upland Game Birds

Table of Contents of Addendum

Discussion	Page
Introduction	i
Response to Public Comments	i
Guideline	
Title/Cover Page	1
Table of Contents	1
I. Summary/Introduction	1
II. Materials/Methods	2
III. Results/Discussion	6
IV. Conclusion	7
V. Tables/Figures	7, 8
VI. Appendix(es)	7, 14

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Avian Reproduction Test for Waterfowl and Upland Game Birds

Subdivision E, Series 71-4

DATA REPORTING

INTRODUCTION

A. Purpose

To provide a data reporting format for an avian reproduction study with waterfowl or upland game birds.

B. Objective

This Data Reporting Guideline is designed to aid the petitioner/registrant in generating reports which are compatible with the Agency's review process. While following this Guideline is not mandatory, data submitters are encouraged to submit complete reports which can be efficiently reviewed by the Agency.

RESPONSE TO PUBLIC COMMENTS

The purpose of this section is to acknowledge and address the concerns expressed in the letters of comment received by the Agency in reponse to the public notice in Federal Register Volume 50, No. 147, p. 31010, July 31, 1985.

This addendum to the Pesticide Assessment Guidelines [Subdivision E] is to be considered an all-encompassing document. The Ecological Effects Branch (EEB) has reviewed the 22 comments submitted by the registrants and/or committees in regard to the Data Reporting Guideline (DRG). This Guideline is not intended to create new data requirements, but to provide for consistent reporting of the necessary avian data required to perform a scientifically sound hazard assessment. This approach should eliminate most, if not all, of the recycling of submissions between EEB scientists and the registrant.

The submitted comments pertain to either items common to all studies or a study specific item. Hence, this discussion has been arranged to consider the general comments first, followed by the specific comments.

General Comments

1. Comments on the location of a specific reporting item -

Considering the need to maintain a consistent format, revisions were made to the DRG in response to these comments wherever possible.

2. Report of the impurities of the test substance -

   Under the present Guideline, these data are required under 158.110 and are reported to the organization reviewing product chemistry submissions. Hence, these data are not reported directly to reviewers of ecological effects studies. Therefore, EEB included item II(A)1.f in this DRG as an option for the registrant. By doing this, a company may avoid any delays which may inadvertently occur in our efforts to obtain the data. To further assure that this item will not be mistaken as a requirement, an explanation has been added to the Guideline.

3. Ingredient information from testing laboratories -

   It appears some sponsors do not make this information available to testing laboratories. In such a situation, the sponsor bears the responsibility since he is submitting the data for registration.

4. Standard evaluation procedures (SEPs) -

   SEPs are guidance documents which explain the procedures used to evaluate environmental effects data submitted to the Office of Pesticide Programs. They are also available from the National Technical Information Service. This DRG is compatible with the Agency's review procedures.

5. Control data -

   The DRG has provided space for reporting results for both control and solvent control results. Both types of controls are commonly reported to the EPA and are also incorporated into the SEPs.

6. Due to the comments, the following items have been deleted from the DRG:

   A. Chemical abstract number or code;
   B. EPA registration number;
   C. Age of material;
   D. Blood testing for a particular test hatch;
   E. Histopathology;
   F. Arrangement of cages in relation to the treatment levels.

7. Environmental conditions -

   Each study reporting item was revised, following the Guideline and SEPs.

8. Pretest diet -

   This is required under §70-4 (c)(D) of the Guideline.

Study Specific Comments

1. The fasting period was deleted as a result of the comments.

2. Tissue analysis -

   One commenter indicated this was a new requirement. However, under Guideline, §71-4(d) Diet Preparation, where bioaccumulation or persistence has been shown by other long-term animal tests, tissue analysis is indicated. Similar analysis is also mentioned in the SEP for this study.

3. Statistical analysis -

   This commenter requested the specific statistical test requirements. The Guideline addresses this question under analysis on page 55 of Subdivision E. However, the statistical analysis portion of the DRG has been reorganized to clarify EEB's needs.

4. Statistical worksheet -

   The DRG was amended to make items of this sort optional. Under "III. RESULTS/DISCUSSION, A. Discuss the results presented in the attached tables." This was added, "Optional graphs, printouts, and other calculations may be attached to the Appendix portion of the report."

5. Table 1b -

   This table was designed to address the Guideline requirements under §71-4(c)(3). However, it is not designed to exclude any additional items or any other methods of data analysis.

6. Table 3 -

   As per a company comment, this table was changed to illustrate the differences in body weight change by sex.

GUIDELINE

The following describes the order and format for a study report.

TITLE/COVER PAGE

Title/cover page and other documents preceeding the study report are discussed in FR 49, 37930.

TABLE OF CONTENTS

I. SUMMARY/INTRODUCTION

A. Sponsor (name of study owner);

B. Name(s) of principal investigator(s);

C. Location of study;

D. Location of raw data and final report;

E. Material (common/trade name);

F. Subject (final or draft report, avian reproduction study - species tested);

G. Test doses (control, concurrent vehicle control,#,#,ppm);

H. Test dates (initiation - date started on treated diet, termination - date of last day of observation);

I. Length of study (days);

J. Results (report chemically related statistical differences and no-observed-effect level);

K. Tested material (chemical name, formulation, percent active ingredient);

L. Test species/strain (both common and scientific name);

M. Source of birds (company, address);

N. Age at initiation of study (days); and

O. Duration of adult exposure prior to egg laying (weeks).

II. MATERIALS/METHODS

This section is a narrative which should include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or the sponsor).

   a. Chemical name;

   b. Composition (qualitative and quantitative description);

   c. Molecular structure;

   d. Percent active ingredient; and

   e. Source, lot number or code.

2. Preparation of test diets (control/treatment).

   a. Report any vehicle used to dissolve or dilute the test substance, or used to mix the test substance with the feed;

   b. Amount of vehicle added to control diet;

   c. Description of test diet and any other ingredients such as antibiotics, vitamins, or food additives which may have been in the feed preceding or during testing;

   d. Water and feed source;

   e. Type of mixer and method of mixing in the test material and vehicle;

   f. Report data if food was analyzed for the pesticide concentration (see Appendix A);

   g. Total volume of material administered (test substance plus carrier) in feed; and

   h. Method of determination of concentration levels.

B. Test Birds

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E.

2. State if test birds:

   a. Phenotypically similar to wild counterpart;

b. Pen-reared; and

c. First breeding season.

3. Description of pretest conditions.

a. Health:

(1) Report birds injured by shipping;

(2) Necropsy findings, if performed;

(3) Sickness;

(4) Mortality;

(5) Abnormalities; and

(6) Chemical and drug exposure.

b. Describe pretest diet (identification and composition including any antibiotics, vitamins, or food additives);

c. Housing conditions:

(1) Number of animals per cage;

(2) Ambient temperature and humidity;

(3) Photoperiod and lighting; and

(4) Source of feed and water.

d. Length of observation.

4. History of colony:

a. Diseases or other medical problems; and

b. Disease treatments.

C. Methods

1. Concentration and dosage mortality data:

a. Number of concentrations;

b. Number of days on test material;

c. Number of animals per cage;

d. Randomization plan for treatments;

e. Method of identifying treatment levels; and

f. Length of observation period.

2. Rationale for selection of method, route, or frequency, if different from that recommended in Subdivision E;

3. A description of the steps taken to maintain the concentration(s), if the test substance is administered in the feed or water. Data from subdivisions other than E, such as Subdivision O or N, suggest that the actual concentration over the length of the test may decrease by 30 percent.

4. Method of assigning test organisms to test and control groups;

5. Experimental design - describe the distribution of birds by dosage, pen, and sex ratio, for example:

(Total number of birds and species) and (## males and ## females) were randomly distributed into the following groups:

Group No.		Dosage Levels ppm	No. of Pens	Birds per Pen	
				Males	Females
1.	Control	0	#	#	#
2.	Solvent control	0	#	#	#
3.	(Test material)	#	#	#	#
4.	(Test material)	##	#	#	#

6. Bird body weights, or other physiological measurements (describe methods, see Table 3b for reporting results);

7. Food consumption:

a. Collection methods (see Table 3b for reporting results);

b. Describe collection schedule; and

c. Provisions for minimizing food spillage and preventing air contamination by volatile chemicals.

8. Describe availability of diets, method and schedule of their renewal;

9. Pen facilities;

a. Pen size and construction:

(1) Space allocations for mating and nesting;

(2) Protection from weather, if outdoors; and

(3) Protection from injuries.

b. Photoperiod and lighting, including hours per day and wattage or footcandles at bird level;

c. Temperature and humidity;

d. Pen location (indoor or outdoor);

e. Diagram of test layout;

f. Ventilation in pen area;

g. Source and availability of water; and

h. Pen construction materials.

10. Weight of fourteen-day-old survivors (see Table 3a);

11. Date egg laying began;

12. Egg collecting, cleaning, and storage procedures;

13. Days eggs candled for eggshell cracks, embryo viability, and embryo survival (see Table 4);

14. Egg rotation regime;

15. Procedure used in selecting eggs for egg shell measurements;

16. Day eggs placed in hatcher and day removed;

17. Method of identifying each bird;

18. Schedule and method for gross necropsy examinations, if performed;

19. List tissue necropsies, if performed;

20. Observation for palatability or repellency of the treated feed;

21. Incubator temperature and humidity;

22. Hatching egg storage temperature and humidity;

23. Describe grouping of hatchlings in relation to treatment levels and parents;

24. Tissue residue data from extra pen group if the test chemical is known to bioaccumulate, if performed (see Appendix B); and

25. Define technical terminology used to describe signs of intoxication.

D. Statistical Analysis

1. Report the statistical method(s) used for analyzing data;

2. Explain any deviations from the referenced method(s);

3. Report the method(s) used to ensure the data meets the assumptions of the referenced statistical method (i.e., a parametric method may require arcsine transformed data);

4. Description of variables analyzed; and

5. Cite references (author, title, journal, number, page, etc.).

III. RESULTS/DISCUSSION

A. Discuss the results presented in the attached tables;

B. If any statistical differences are found discuss their biological significance;

C. Describe and discuss control bird reproduction parameters, behavior, and general health which appear atypical;

D. Detailed description of nature, incidence, time of occurrence, severity, and duration of all observed toxic effects including all morphological and physiological responses, death and any other abnormal or unusual signs and symptoms. For example, report any specific signs of intoxication associated with death and the time of onset and remission of toxic signs. Also, report recovery time for affected birds. In addition, marked observations of fecal material and urine should be reported.

E. Any gross pathological changes as noted by observation or gross necropsy examination including characterization of gross lesions;

F. Report repellency of the diet or lack of palatability;

G. Provide no-observed-effect-level;

H. Report any other items related to the study which may be of value in hazard assessment or evaluation of the study;

I. Indicate the test procedures which deviate from the EPA Guideline and provide a rationale for the change;

J. Report and discuss the results of residue analysis of test diet (see Appendix); and

K. Degree of bioaccumulation if this portion of the study is performed.

IV. CONCLUSION

This is a summation of the above Results/Discussion section.

V. TABLES/FIGURES

VI. APPENDIX(ES)

This is optional. You may attach graphs, raw data, printouts, calculations, etc., which may further support the study.

TABLE 1a

Reproductive Data - (Test Species)

	Control	Compound (Test Material Name) (ppm) #.#	##.#
Eggs Laid	###	###	###
Eggs Cracked	##	##	##
Eggs Set	###	###	###
Viable Embryos	###	###	###
Live Three-Week Embryos	###	###	###
Normal Hatchlings	###	###	###
14 Day-Old Survivors	###	###	###

TABLE 1b

Reproductive Success Data - (Test Species)

	Control	Compound (Test Material Name(ppm) #.#	##.#
Eggs Laid Per Hen In Eight Weeks	##.#	##.#	##.#
Eggs Cracked of Eggs Laid (%)	#.#	##.#	##.#
Viable Embryos of Eggs Set	##.#	##.#	##.#
Live Three-Week Embryos of of Viable Embryos (%)	##.#	##.#	##.#
Normal Hatchling of Live Three-Week Embryos (%)	##.#	##.#	##.#
14 Day-Old Survivors of Normal Hatchlings	##.#	##.#	##.#
14 Day-Old Survivors of Per Hen	##.#	##.#	##.#

TABLE 2

Eggshell Thickness Data - (Test Species)

	CONTROLS	(Test Material ppm)	
		#.#	##.#
No. of Eggs Analyzed	##	##	##
Mean Shell Thickness	#.###	#.###	#.###

TABLE 3a

Weights(grams)of Fourteen - Day - Old Survivors

BIRD#	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
CONTROL	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
TEST DIET (ppm)	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##
TEST DIET (ppm)	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##	##

TABLE 3b

Body Weight and Feed Consumption Data - (Test Species)

WEEK	CONTROLS B.W. Male	CONTROLS B.W. Female	CONTROLS F.C.*	Test (Material Name) B.W. Male	Test (Material Name) B.W. Female	Test (Material Name) F.C.*	Test (Material Name) B.W. Male	Test (Material Name) B.W. Female	Test (Material Name) F.C.*
Init.	###	###	—	###	###	—	###	###	—
1	--	--	##	--	--	##	--	--	##
2	###	###	—	###	###	—	###	###	—
3	--	--	##	--	--	##	--	--	##
4	###	###	—	###	###	—	###	###	—
5	--	--	##	--	--	##	--	--	##
6	###*	###	—	###	###	—	###	###	—
7	--	--	##	--	--	##	--	--	##
8	###*	###	—	###	###	—	###	###	—
9	--	--	##	--	--	##	--	--	##
10	--	--	—	--	--	—	—	--	—
11	--	--	##	--	--	##	--	--	##
12	--	--	—	--	--	—	—	--	—
13	--	--	##	--	--	##	--	--	##
14	--	--	—	--	--	—	—	--	—
15	--	--	##	--	--	##	--	--	##
16	--	--	—	--	--	—	—	--	—
17	--	--	##	--	--	##	--	--	##
18	###	###	—	###	###	—	###	###	—

The body weight data are presented as a group mean.
The food consumption data are presented as the group mean feed consumed per bird per day.

B.W. - Body Weight
F.C. - Food Consumption
* - Feed consumption was for the entire two-week period.

TABLE 4

REPRODUCTIVE DATA BY PEN - (Test Species)

(Test Material Name)

	Eggs Laid	Eggs Cracked	Eggs Set	Viable Embryos	Live Three-Week Embryos	Normal Hatchlings
Controls	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	##	##	##	##	##
	##	#	##	##	##	##
	##	##	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
Totals	###	##	###	###	###	###
(Treatment)	##	#	##	##	##	#
	#	#	#	#	#	#
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	##	##	##	##	##
	##	##	##	##	##	##
	##	##	##	##	##	##
Totals	###	##	###	###	###	###

(continued)

REPRODUCTIVE DATA BY PEN - (Test Species)

(Test Material Name)

	Eggs Laid	Eggs Cracked	Eggs Set	Viable Embryos	Live Three-Week Embryos	Normal Hatchlings
(Treatment)	##	#	##	##	##	#
	##	#	##	##	#	#
	##	##	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	##	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	##	##
	##	#	##	##	#	#
Totals	###	##	###	###	###	###

TABLE 5

14-DAY-OLD SURVIVORS BY WEEK - (Test Species)

(Test Material Name)

Week #	1	2	3	4	5	6	7	8	Totals
Controls	##	##	##	##	##	##	##	##	###
# ppm	##	##	##	##	##	##	##	##	###
## ppm	##	##	##	##	##	##	##	##	###

SHELL THICKNESS DATA BY PEN - (Test Species)

(Test Material Name)

	(Thickness Measured in Millimeters)										Average
Controls	.###	.###	.###	.###	.###	.###	.###	.###	.###	.###	.###
# ppm	.###	.###	.###	.###	.###	.###	.###	.###	.###	.###	.###
## ppm	.###	.###	.###	.###	.###	.###	.###	.###	.###	.###	.###

APPENDIX A

RESULTS OF ASSAYS

MEASURED DIETARY CONCENTRATIONS OF (Test Material Name) IN DIETS USED DURING (Test Species) ONE-GENERATION REPRODUCTION STUDIES.

(Laboratory Name Number or Code)

Date Prepared	Dated Collected	Date Assayed	Nominal Concentrations (ppm)	Measured Concentrations (ppm)
##-##-##	##-##-##	##-##-##	0	(NDR)[a]
			#	#.#
			##	##.#
##-##-##	##-##-##	##-##-##	0	(NDR)[a]
			#	#.#
			##	##.#
##-##-##	##-##-##	##-##-##	0	(NDR)[a]
			#	#.#
			##	##.#
##-##-##	##-##-##	##-##-##	0	(NDR)[a]
			#	#.#
			##	##.#
##-##-##	##-##-##	##-##-##	0	(NDR)[a]
			#	#.#
			##	##.#

[a] NDR = No Detectable Residue.

APPENDIX B

BIRD RESIDUE ANALYSIS RESULTS OF (Test Material)
FOR (Test Species) ONE-GENERATION REPRODUCTION STUDY

(Laboratory Name, Number or Code)

Test Dosage	Bird#	Date Assayed	Results	
			Tissue	Concentration
##	##	##-##-##	*******	##.#
	"	"	"	"
	"	"	"	"
	"	"	"	"
	"	"	"	"
##	##	##-##-##	*******	##.#
	"	"	"	"
	"	"	"	"
	"	"	"	"
	"	"	"	"